CARDIOVASCULAR MEDICINE

Haemochromatosis gene mutations and risk of coronary heart disease: a west of Scotland coronary prevention study (WOSCOPS) substudy

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Objectives: To measure the frequency of genotypes of the HFE (haemochromatosis) gene in patients recruited to the west of Scotland coronary prevention study (WOSCOPS), and relate them to the subsequent occurrence of coronary clinical events.
Design: Nested case–control study, drawing samples of DNA from the biological bank of a cohort study.
Patients: Men aged 45–64 years in 1989, with moderate hypercholesterolaemia and no evidence of coronary heart disease at baseline.
Interventions: Follow up for a mean period of 4.9 years. Typing for C282Y and H63D mutations of the HFE gene in 482 subjects with a subsequent coronary event and 1104 without an event.
Results: The C282Y mutation was present in 81 of 482 cases (16.8%) and 182 of 1104 controls (16.5%). Comparing the prevalence of gene mutations in the cases and controls, there were no significant differences. The hazard ratio for C282Y heterozygotes was 1.03 (95% confidence interval (CI) 0.77 to 1.36) and for C282Y/H63D compound heterozygotes 1.04 (95% CI 0.50 to 2.14). Prespecified subgroup analyses of the pravastatin, placebo, smoking, and non-smoking groups showed no significant differences between cases and controls. Repeating the analyses after adjusting for possible confounding factors produced no change in the results.
Conclusions: In a population of moderately hypercholesterolaemic middle aged Scottish men who did not have any evidence of coronary heart disease at baseline, the presence of a C282Y mutation in the HFE gene did not predict the occurrence of coronary events over a mean follow up of 4.9 years.

There has been debate for some years about the possibility that iron excess is a risk factor for coronary heart disease, and that iron depletion is protective.[1] A postulated mechanism is that excess body iron increases free radical mediated oxidative stress either in the circulation, the vessel wall, or the myocardium, although the development of glucose intolerance and diabetes is a further possibility.[2]

Blood donors have a lower risk of coronary heart disease than non-donors, and although this may be expected because they are in relatively good health before they are permitted to donate, recent studies have shown this protective effect when both the donor and non-donor population were in similar states of health at entry to the study.[3 4] It is well recognised that women, whose body iron stores are lower than men's, have lower rates of coronary heart disease.

In the mid 1990s, the relation between body iron stores and coronary heart disease remained unclear because of the conflicting evidence arising from different study designs.[5] In 1996 the gene mutation responsible for over 90% of cases of genetic haemochromatosis was identified.[6] The population frequency of this C282Y mutation in the HFE gene appears to be greatest in northern Europe; in Scotland the gene frequency has been reported to be 8.6%,[7] while in Spain, Italy, and Greece it is of the order of 0.5% to 3.2%.[8]

A Medline search using combinations of key words ''iron'', ''h(a)emochromatosis'', ''coronary heart disease'', and ''myocardial infarction'' revealed several published studies that have addressed the possible linkage of C282Y to coronary heart disease. Seven case–control studies have failed to show a link,[9–15] while three cohort studies have suggested that heterozygotes for the mutation have a significantly increased risk of coronary heart disease, particularly when there are other risk factors present such as smoking and hypertension.[16–18]

A potential genetic risk factor that is present in 10% of the population, and which should be modifiable by the simple procedure of depletion of body iron, is clearly important. In west Scotland, there is a population of middle aged men who have been thoroughly documented in the west of Scotland coronary prevention study (WOSCOPS). These men were selected for study because they had moderate hypercholesterolaemia; they were then randomised to pravastatin or placebo treatment and followed up for an average of 4.9 years, with documentation of the occurrence of fatal and non-fatal coronary events, death from cardiovascular disease, and death from all causes.[19] A Biobank has been set up containing DNA from all individuals who had a coronary event in this study, and from two controls matched for age and smoking habits. The WOSCOPS executive agreed to release this DNA material for analysis of mutations in the HFE gene. No remaining serum samples were available for iron, transferrin, or ferritin analyses. We aimed to measure the frequency of genotypes of the HFE gene in patients recruited to the WOSCOPS study, and relate them to the subsequent occurrence of coronary clinical events.

METHODS

The subjects included were men aged 45–64 years in 1989 who had blood samples as part of the WOSCOPS protocol.

Abbreviations: ARIC, atherosclerosis risk in communities; KIHD, Kuopio ischaemic risk factor study; WOSCOPS, west of Scotland coronary prevention study

Clinical data were available in 580 cases who subsequently had a coronary event (fatal or non-fatal myocardial infarction in 503, revascularisation procedure in 77), and 1160 matched controls who had not had a coronary event, over a mean follow up period of 4.9 years. Specimens for genetic analysis had been stored in a Biobank at −70°C. For the current study, specimens were missing or insufficient to allow both C282Y and H63D polymorphism analysis from 98 patients who had a coronary event (16.9%) and from 56 subjects who had no coronary event (4.8%). The reason for the higher frequency of missing specimens in the cases is not known, but was not regarded as a significant source of bias.

On specimens from the remaining 482 cases and 1104 controls, C282Y and H63D polymorphisms of the HFE gene were tested for by polymerase chain reaction and restriction enzyme digest[6] followed by separation of the fragments on a polyacrylamide gel.

Approval for the project was granted by the Lanarkshire and North Glasgow Trust ethics and research committees.

Statistics

Statistical analysis was undertaken using conditional logistic regression analysis, with the results presented in the form of odds ratios and 95% confidence intervals (CI). The study was designed to have a power of 80% and two tailed significance of 5% to detect odds ratios of 1.53 for the C282Y heterozygous state and 1.93 for the C282Y/H63D compound heterozygous state, compared with the wild type homozygous states. Odds ratios were calculated by univariate analysis, and again after adjustment for possible confounding factors—body mass index, blood pressure, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, fibrinogen, white cell count, and C reactive protein.

RESULTS

The baseline characteristics of the cases and controls in the present study are shown in table 1. Subjects who experienced a coronary event during the 4.9 years of trial follow up had a higher body mass index, blood pressure, LDL cholesterol, fibrinogen, white cell count, and C reactive protein, a lower HDL cholesterol, and were more likely to have hypertension.

In the 1104 control samples analysed, 922 (83.5%) did not carry the C282Y mutation, 179 (16.2%) were heterozygotes for C282Y, and three (0.3%) were C282Y homozygotes. Twenty five subjects (2.3%) were compound heterozygotes for C282Y/H63D. The frequency of C282Y homozygotes is less than would be expected given the frequency of the C282Y heterozygotes (χ^2, $p = 0.063$). The likely explanation for this is that some C282Y homozygotes may have been excluded from entry into the WOSCOPS study because of abnormalities of liver function tests.

Table 2 HFE genotypes in cases and controls

	Cases (n = 482)	Controls (n = 1104)
Homozygous wild type	401 (83.2)	922 (83.5)
C282Y heterozygote	80 (16.6)	179 (16.2)
C282Y/H63D compound heterozygote	11 (2.3)	25 (2.3)
C282Y homozygote	1	3

Figures in parentheses are percentages.

Table 2 shows the frequency of HFE genotypes in cases and controls. The distributions were nearly identical. Table 3 summarises hazard ratios and their 95% confidence intervals (CI) in prespecified subgroup analyses. Again there were no significant differences between cases and controls. Repeating the analyses after adjusting for possible confounding factors (body mass index, blood pressure, LDL and HDL cholesterol, white cell count, fibrinogen, and C reactive protein) gave an odds ratio of 0.87 (95% CI 0.63 to 1.19) for C282Y heterozygotes and 0.39 (95% CI 0.13 to 1.17) for C282Y/H63D compound heterozygotes. These were non-significant differences, although the trend is towards a protective effect of the C282Y mutation for coronary heart disease.

DISCUSSION

The C282Y mutation in the HFE gene was present in heterozygous state in 16.2% of control subjects in this sample of Scottish middle aged men with moderate hypercholesterolaemia. This is consistent with the high prevalence of the mutation previously noted in healthy northern European populations.

Our results, using a nested case–control design drawing samples from a cohort study, do not show evidence of a link between C282Y heterozygosity and coronary events. This finding is consistent with the reports of several point case–control studies which show no such association.[9–15]

Three published cohort studies have suggested an increased risk of coronary events in C282Y heterozygotes. The Finnish Kuopio ischaemic risk factor study (KIHD)[16] reported on men aged 42–60 years with no coronary heart disease at baseline. Over a mean follow up of nine years there were 68 subjects with acute myocardial infarction, eight (11.8%) of whom were C282Y heterozygotes. Seventy seven

Table 1 Baseline characteristics of cases and controls

Variable	Cases (n = 483)	Controls (n = 1107)	p Value	Original study cohort (n = 6595)
Age (years)	56.9 (5.1)	56.7 (5.2)	0.68	55.2 (5.5)
Body mass index (kg/m^2)	26.0 (3.1)	25.6 (3.2)	0.03	26.0 (3.2)
Systolic BP (mm Hg)	139 (17)	136 (17)	<0.001	136 (17)
Diastolic BP (mm Hg)	85 (10)	84 (10)	0.002	84 (10)
Cholesterol (mmol/l)	7.07 (0.61)	7.02 (0.58)	0.10	7.03 (0.59)
LDL cholesterol (mmol/l)	5.02 (0.46)	4.95 (0.44)	0.005	4.97 (0.44)
HDL cholesterol (mmol/l)	1.07 (0.22)	1.14 (0.25)	<0.001	1.14 (0.26)
Fibrinogen (g/l)	4.52 (0.87)	4.35 (0.86)	<0.001	–
White cell count (×10^9/l)	7.06 (1.89)	6.75 (1.86)	0.002	–
C reactive protein (mg/l)	3.88 (4.68)	3.29 (4.46)	<0.001	–
Smoker (%)	53.7	54.4	0.80	44.1
Diabetes (%)	2.1	1.2	0.17	1.2
Hypertension (%)	23.4	15.6	<0.001	15.7

Values are mean (SD) unless stated. Cases and controls were matched for age and smoking status.
The p values are for comparison between the case group and control group. The two sample *t* test was used for continuous variables and χ^2 test for categorical variables. The p value for C reactive protein was derived from a logarithmic analysis. The characteristics of the entire WOSCOPS cohort are given for comparison. Formal analysis of these variables to predict coronary heart disease risk has been published elsewhere.[19]
BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3 Relative risk of HFE genotypes for coronary events in subgroups

	C282Y heterozygote	C282Y/H63D compound heterozygote
Total (n = 1586)	1.03 (0.77 to 1.37)	1.04 (0.50 to 2.14)
Placebo (n = 827)	0.93 (0.64 to 1.36)	0.96 (0.40 to 2.30)
Pravastatin (n = 759)	1.18 (0.76 to 1.84)	1.17 (0.30 to 4.49)
Non-smokers (n = 726)	0.88 (0.57 to 1.35)	1.16 (0.43 to 3.16)
Smokers (n = 860)	1.18 (0.80 to 1.73)	0.93 (0.33 to 2.64)

The values shown are the odds ratios for coronary events for C282Y heterozygotes and C282Y/H63D compounds heterozygotes compared with wild type homozygotes. Figures in parentheses are 95% confidence intervals.

(6.7%) of 1150 subjects who did not experience acute myocardial infarction were C282Y heterozygotes. The crude relative risk of acute myocardial infarction was 2.0 (95% CI 0.9 to 4.1) and the relative risk adjusted for other risk factors was 2.3 (95% CI 1.1 to 4.8).

In the second study,[17] a cohort of 12239 Dutch women aged 51–69 years at baseline was followed for 16–18 years; C282Y status was assessed among 531 women who died of cardiovascular disease and 555 randomly selected women who did not die from this cause. The number of deaths from acute myocardial infarction is not stated, but a 1.5-fold increased risk (95% CI 0.9 to 2.5) was reported for C282Y heterozygotes.

The third study was the US ARIC (atherosclerosis risk in communities) study,[18] which recruited 14 215 subjects aged 45–64 years at baseline (43% male, 57% female; 25% black, 75% white) and followed them up for a mean of 2.9 years. Over this period, there were 243 incident cases of coronary heart disease (157 probable or definite myocardial infarctions, 20 silent myocardial infarctions, 26 definite fatal coronary heart disease, 40 revascularisation procedures), and a reference cohort of 535 subjects was identified. The crude relative risk of coronary heart disease in C282Y heterozygotes was 1.6 (95% CI 0.9 to 3.0), and 2.7 (95% CI 1.2 to 6.0) after controlling for other risk factors such as lower prevalence of smoking and lower mean LDL cholesterol in the C282Y group.

The reason why three cohort studies have suggested that the C282Y mutation is a risk factor for coronary heart disease, while our study and several case–control studies have shown no evidence of a link, is not immediately clear. The results in the positive cohort studies were of marginal statistical significance, with confidence intervals crossing the line of unity only after adjustment for possible confounding factors. In the case–control studies, it is possible that survival bias contributed to the negative findings, but the three prospective studies did not specifically report a stronger impact of C282Y heterozygosity on fatal coronary heart disease. In our study there was a follow up of 4.9 years, and it might be argued that this was not long enough to allow iron to accumulate in sufficient amounts to have an adverse effect on free radical mediated oxidative stress. However, the age group we studied was similar to that of the other three prospective studies, and our population was exclusively male (men are known to have higher serum iron concentrations than women). It is possible that the hypercholesterolaemic nature of our study group resulted in a masking of a true association between C282Y and coronary heart disease. However, if the oxidative stress mechanism proposed for a true association were correct, one might expect a greater likelihood of finding a significant relative risk in subjects with high LDL cholesterol values.

We did not have access to serum samples to measure iron, transferrin, and ferritin concentrations in our subjects. In published work, two of eight prospective cohort studies have reported an association of serum ferritin with risk of coronary heart disease or atherosclerosis, and none of five cohort studies assessing transferrin saturation has reported an association with increased risk of coronary heart disease.[20 21]

Taken as a whole, the evidence linking C282Y heterozygosity as a risk factor for coronary events is tenuous. Our study provides further evidence that no such link exists.

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